sobi
Pioneer in Rare Diseases

Patrik Strömberg PhD MBA

Senior Director, Head of Biomedical Science & Portfolio Innovation, Swedish Orphan Biovitrum AB (Sobi)

Biopharmaceuticals - an introduction

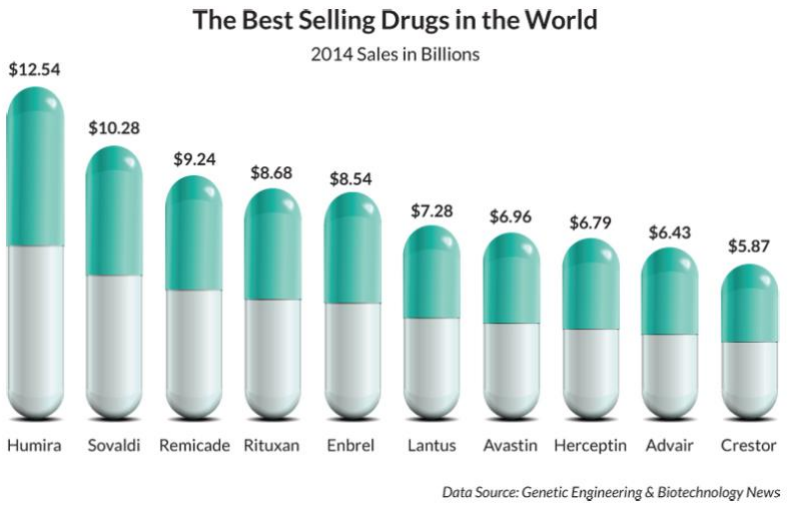
KTH, September 5, 2018, Stockholm

Outline

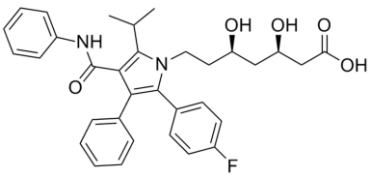
sobi

- Introduction
  - Proteins and protein drugs
  - Biotechnological production
  - Gene technology and protein engineering
  - Monoclonal antibodies
- Classification of protein drugs
- Differences between protein- and small molecular drugs
- Important aspects and opportunities for protein drugs
  - Example 1. EPO
  - Example 2. TNF blockers

2

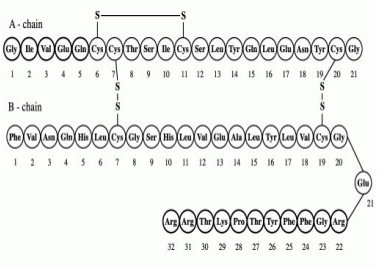


3



Formula	$C_{33}H_{35}FN_2O_5$
Mol. mass	559 g/mol

4



Formula	$C_{267}H_{408}N_{72}O_{77}S_6$
Mol. mass	6 063 g/mol

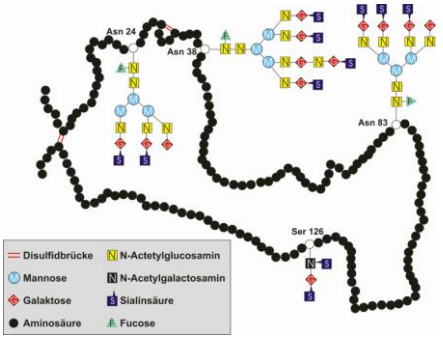
5

Proteins and protein drugs

- Large macromolecules (>5 kDa)
  - 10 – 1000 x traditional small molecule drugs
  - (Glyco)proteins consisting of 20 different L-amino acids and sugar groups (and other modifications)
- Protein drugs
  - Complex structures enabling complex functions
    - Enzymes, growth factors, multiple interactions
  - Large pharmacophore
    - Specificity and potency
- Terminology
  - Biologic(al)s (biotherapeutics), Biopharmceuticals, Biotechnology-derived pharmaceuticals or protein pharmaceuticals
    - Some names also include blood products, antitoxins, vaccines, nucleic acids (DNA vaccine, antisense, gene therapy) etc.
  - NME – new molecular entity

1923 Insulin purified from pigs and cows is introduced (Eli Lilly)
1982 Humant recombinant Insulin produced in *E.coli* (Genentech /Eli Lilly)

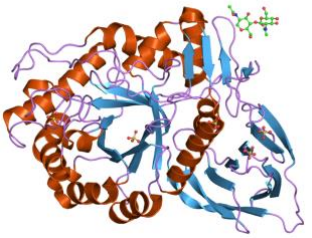
6



Formula	$C_{815}H_{1317}N_{233}O_{241}S_5$
Mol. mass	18 396 g/mol *

* Incl. Glycosylation = 37 kDa

7



Formula	$C_{2532}H_{3854}N_{672}O_{711}S_{16}$
Mol. mass	55597.4 g/mol (unglycosylated)

Enzymes are proteins that catalyze chemical reactions (i.e. increase the reaction velocity). Almost all biological processes within the cell need enzymes to proceed at appreciable rates.

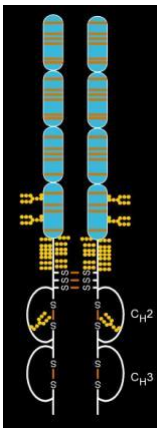
8

Biotechnological production

- Most protein drugs are produced using recombinant DNA technology
  - Cloning of gene and/or cDNA
  - DNA is introduced into host cells (cell line)
  - Heterologous protein expression
- Frequently used expression systems:
  - Microorganisms: bacteria (eg. *E.coli*) and yeast
  - Eukaryotic cells: insect cells, mammalian cells (eg. CHO)
  - Transgenic animals and plants
- Protein/process characterization
  - Complex structures, patterns of posttranslational modifications
  - Product- and/or process-specific impurities
  - Important to be able to show:
    - Identity, Purity, Stability and Consistency of manufacture




9



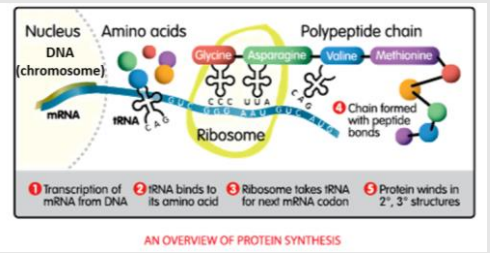
Formula	$C_{2224}H_{3475}N_{621}O_{698}S_{36}$
Mol. mass	51 235 g/mol*

* Dimer + glycosylation = 150 kDa

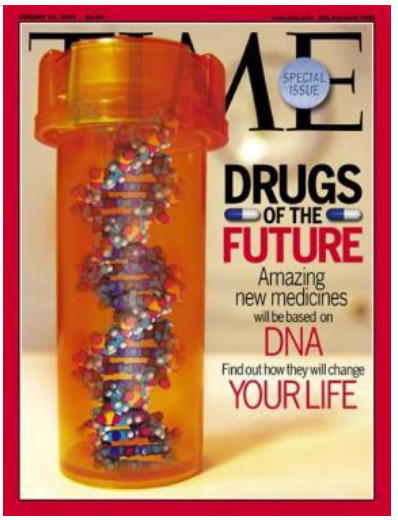
10

Gene technology and protein engineering

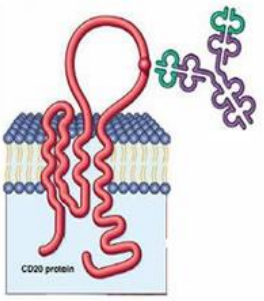
- ”Cut-and-paste” DNA
- Design and modification of proteins
- Fusions and chimeric molecules
- Production by biotechnological processes



AN OVERVIEW OF PROTEIN SYNTHESIS



11

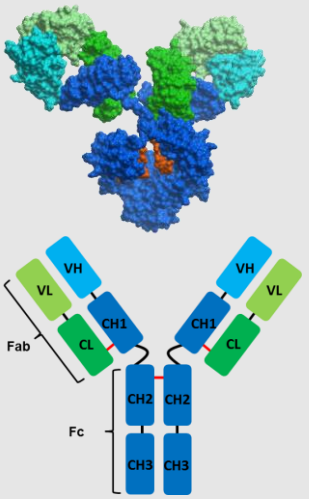


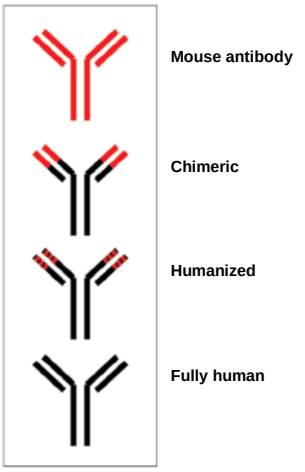
Formula	$C_{6416}H_{9874}N_{1688}O_{1987}S_{44}$
Mol. mass	143 860 g/mol

12

Monoclonal antibodies (mAbs)

- Antibodies as drugs
  - Targeting
    - Variable regions (CDRs)
  - Stability and effector functions
    - Fc region
  - mAbs are usually produced by immunizations in mice
    - Immunogenicity
    - Humanization
  - Fully-human mAbs
    - Transgenic mice
    - In vitro selection
  - Either full-length antibodies or antibody fragments (and fusions) can be used
    - Antibody engineering





Isaacs JD, Arthritis Research & Therapy, 2009

13

Table 1 Comparison of major technology platforms for therapeutic antibody discovery

Platforms	Main applications	Major advantages	Key disadvantages	FDA approved therapeutic antibodies
Hybridoma	Generate hits and research reagents	Mature technology and cost effective	Potential immunogenicity	Orthoclone, Zevalin, Bexxar
Humanization	Generate therapeutic candidates	Well established and low cost	Not fully human antibodies	ReoPro, Rituxan, Simulect, Remicade, Erbitux, Adcetris, Zenapax, Synagis, Herceptin, Mylotarg, Mabcampath, Xolair, Actemra, Avastin, Tysabri, Lucentis, Soliris, Cimzia, Perjeta
Phage display	Generate hits and therapeutic candidates	Large library size (> 10E10) and robust screening; fully human antibodies	Not all antibodies express well in Escherichia coli and require engineering	Humira and Benlysta
Yeast display	Improve affinity and stability	Eukaryotic host; targeted sorting by FACS	Relatively small library size	None
Transgenic Rodents	Generate therapeutic candidates	High affinity fully human antibodies	Technology accessibility	Vectibis, Ilaris, Simponi, Stelara, Arzerra, Prolia, Yervoy

FDA: Food and Drug Administration; FACS: Fluorescence activated cell sorting.

Lu et al. World J Biol Chem 2012 December 26; 3(12): 187-196

Antibody-based biopharmaceuticals

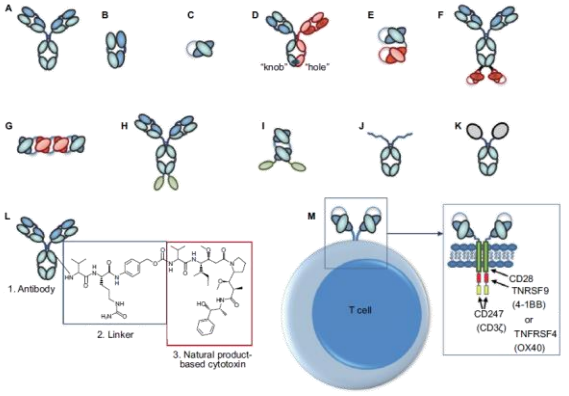


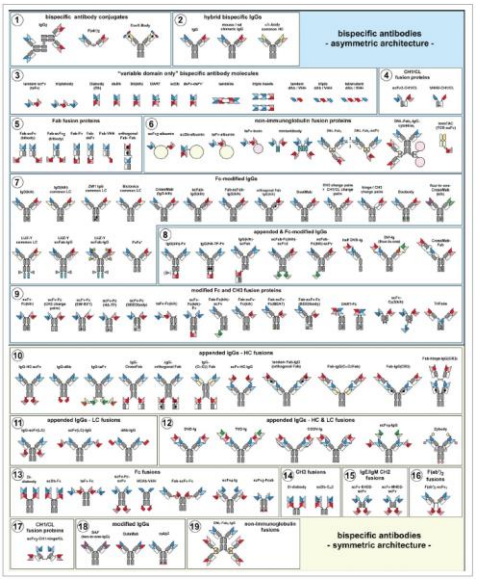
Table 3. Current status of innovative antibody, Fc fusion protein, and chimeric antigen receptor (CAR) drug candidates*

Antibody format	Stage of development			Totals
	Phase I/II	Phase III	Approved for marketing at some point**	
Naked IgG	30	51	52	493
Naked antibody fragments	7	2	4	13
Immunocytokines	9	2	0	11
Fc fusion proteins	23	3	11	37
Bispecific antibodies	58	1	2	61
• IgG-like	• (41)	• (1)	• (1)	• (43)
• Fragment-based	• (14)	• (0)	• (1)	• (15)
• Nanoparticle***	• (03)	• (0)	• (0)	• (03)
Antibody-drug conjugates#	75	9	3	87
Radioimmunoglobulins	13	2	2	17
Antibodies only	575	70	74	719
T or NK cells expressing CAR antibodies	145	0	0	145
Totals	720	70	74	864

Abbreviations: IgG, immunoglobulin G; CAR, chimeric antigen receptor.

Strohl WR, Protein Cell 2017

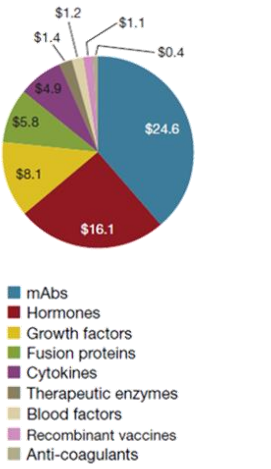
The zoo of bispecific antibody formats



Brinkmann U & Kontermann RE, mAbs 2017

Classification of biopharmaceuticals

- Substitution therapy:
  - Replacement of defective/absent protein
  - Augmentation of signaling pathway
    - Insulin, growth hormone, coagulation factors, enzyme replacement
    - Haematopoiesis (Epo), Stimulation of white blood cells (G-CSF), fertility hormone treatment (FSH), immune regulation (interferon)
- Targeting
  - Antibodies or related molecules/scaffolds
  - Natural receptors or regulatory molecules
    - Cancer targeting
    - Immuno inflammation, autoimmunity
    - Immuno oncology
    - Etc.



Aggarwal RS, Nature Biotechnology, 2014

17

Differences between small molecules and biopharmaceuticals

	Small molecules	Protein drugs
Targets	Intra- and extracellular (entire body)	Extracellular (limited distribution)
Specificity	Often broad with activity in several species	Often limited to human and monkey
Preclinical studies	Generic (guidelines)	Alternative, case-by-case (surrogate molecules etc.)
Safety and tox	Off-target toxicity and reactive metabolites	On-target / off-pathology, immunogenicity
Administration	Oral (same throughout development)	Injection (i.v. infusion or s.c.)
DMPK	Short duration, drug metabolism	Long half-life desirable (less injections), catabolism
Manufacturing (process, scale)	Chemical synthesis, often same process (upscaling)	Biotechnological process, common to change process/scale, costly
Purity and stability	High purity (absence of toxic impurities) and high stability	Structural heterogeneity, sensitivity to high temperature and long-term storage

18

Important properties, considerations, and opportunities for biopharmaceuticals

- Manufacturing costs
  - Expression systems
  - Expressions levels
  - Process (upstream, downstream, analysis, formulation)
- Immunogenicity
  - Efficacy, safety, pharmacokinetics, pharmacodynamics
  - Humanization, glycosylation, host cell
- Dosage and pharmacokinetics
  - Route of administration: i.v., i.m., s.c.
  - Pharmacokinetics: plasma half-life, bioavailability, ADME
  - Dose regimen
  - "Convenience"
- Intellectual property rights
  - Freedom-to-operate and product protection
  - Biosimilars

19

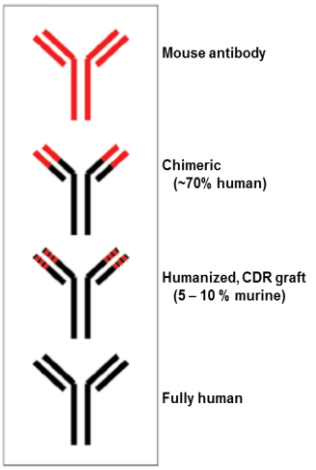
Important properties, considerations, and opportunities for biopharmaceuticals

- Manufacturing costs
  - Expression systems
  - Expressions levels
  - Process (upstream, downstream, analysis, formulation)
- Immunogenicity
  - Efficacy, safety, pharmacokinetics, pharmacodynamics
  - Humanization, glycosylation, host cell
- Dosage and pharmacokinetics
  - Route of administration: i.v., i.m., s.c.
  - Pharmacokinetics: plasma half-life, bioavailability, ADME
  - Dose regimen
  - "Convenience"
- Intellectual property rights
  - Freedom-to-operate and product protection
  - Biosimilars

20

Important properties, considerations, and opportunities for biopharmaceuticals

- Manufacturing costs
  - Expression systems
  - Expressions levels
  - Process (upstream, downstream, analysis, formulation)
- Immunogenicity
  - Efficacy, safety, pharmacokinetics, pharmacodynamics
  - Humanization, glycosylation, host cell
- Dosage and pharmacokinetics
  - Route of administration: i.v., i.m., s.c.
  - Pharmacokinetics: plasma half-life, bioavailability, ADME
  - Dose regimen
  - "Convenience"
- Intellectual property rights
  - Freedom-to-operate and product protection
  - Biosimilars



21

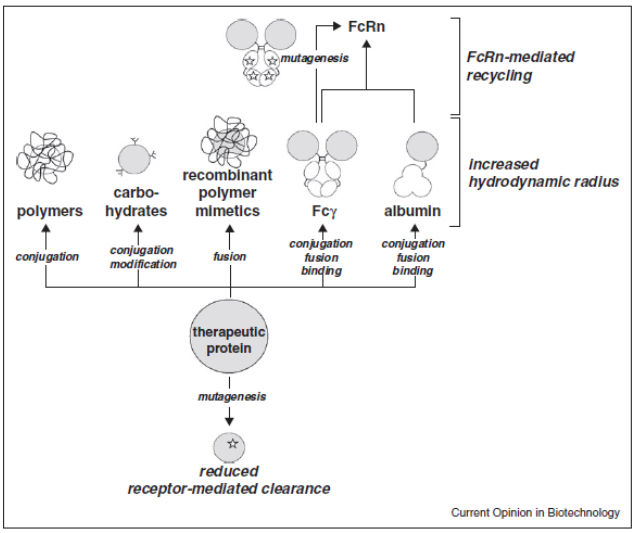
Important properties, considerations, and opportunities for biopharmaceuticals

- Manufacturing costs
  - Expression systems
  - Expressions levels
  - Process (upstream, downstream, analysis, formulation)
- Immunogenicity
  - Efficacy, safety, pharmacokinetics, pharmacodynamics
  - Humanization, glycosylation, host cell
- Dosage and pharmacokinetics
  - Route of administration: i.v., i.m., s.c.
  - Pharmacokinetics: plasma half-life, bioavailability, ADME
  - Dose regimen
  - "Convenience"
- Intellectual property rights
  - Freedom-to-operate and product protection
  - Biosimilars

22

Measures to extend the plasma half-life are important for improving drug-like properties of biopharmaceuticals

- Clearance of protein drugs from circulation
  - Blood mediated elimination by proteolysis
  - Renal filtration and degradation
  - Hepatic elimination
  - Elimination by receptor-mediated endocytosis
- Molecules with a small size or low molecular mass are rapidly cleared by renal filtration
  - Threshold in the range of 40–50 kDa
  - Hydrodynamic radius and physicochemical properties
- Protein engineering techniques can dramatically decrease clearance
  - Genetic fusions
  - Chemical conjugation and modification
  - Site-specific mutagenesis
- Recycling through neonatal Fc receptor (FcRn)



Kontermann RE *Current Opinion in Biotechnology* 2011

23

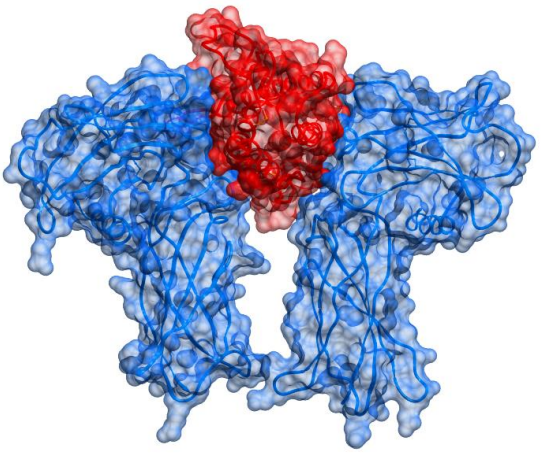
Important properties, considerations, and opportunities for a protein drug

- Manufacturing costs
  - Expression systems
  - Expressions levels
  - Process (upstream, downstream, analysis, formulation)
- Immunogenicity
  - Efficacy, safety, pharmacokinetics, pharmacodynamics
  - Humanization, glycosylation, host cell
- Dosage and pharmacokinetics
  - Route of administration: i.v., i.m., s.c.
  - Pharmacokinetics: plasma half-life, bioavailability, ADME
  - Dose regimen
  - "Convenience"
- Intellectual property rights
  - Freedom-to-operate and product protection
  - Biosimilars

24

Example 1. Erythropoietin (Epo)

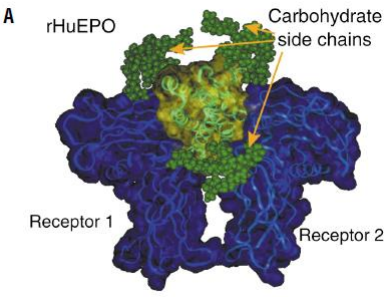
- Glycoprotein hormone (growth factor) that regulates the production of erythrocytes
- Naturally secreted from the kidneys
- Recombinant Epo has been in use for more than 25 years
  - Improved novel Epo-variants now available
  - Epo-doping
- Epo is used to treat anemia as a result of renal failure (or e.g. cancer treatment)



- 1989: the first Epo drug was approved
  - Epoeitin alfa (Epogen/Prokrit)
  - Recombinant protein based on the human Epo sequence
    - 165 aa (3 glycosylations), 30 kDa
  - Produced in CHO cells (hamster origin)
  - **Short plasma half-life (~ 8 h)**
    - **Three doses / week**

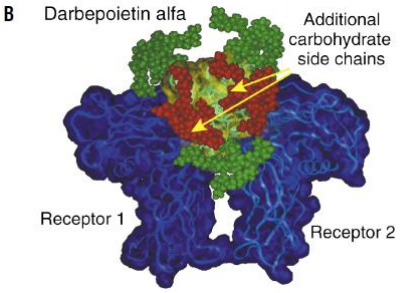
Use of Epo has been associated with Pure Red Cell Aplasia (PRCA) – a rare type of severe anemia.

A result of Anti-Epo antibodies (immunogenicity)



Elliott S et al. *Nature Biotechnology* 2003

- 2001: a new Epo drug with improved pharmacokinetics was launched
  - Darbepoetin (Aranesp)
  - Hyperglycosylated
    - 5 glykosylations, 37 kDa
  - **Prolonged half-life (25 h)**
    - **One dose / week**



Elliott S et al. *Nature Biotechnology* 2003

- 2001: a new Epo drug with improved pharmacokinetics was launched
  - Darbepoetin (Aranesp)
  - Hyperglycosylated
    - 5 glykosylations, 37 kDa
  - **Prolonged half-life (25 h)**
    - **One dose / week**

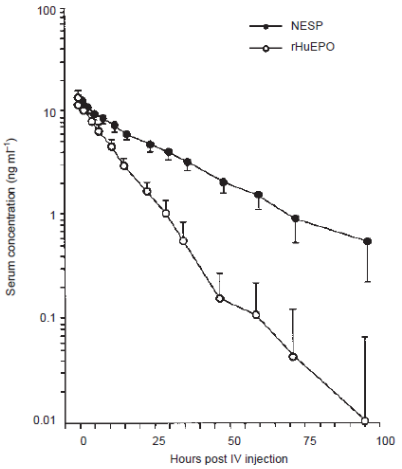


Figure 9 Comparative pharmacokinetics of NESP and epoetin alfa in anaemic dialysis patients. Results are expressed as the mean (± SD). Reproduced by the kind permission of Lippincott Williams and Wilkins from Macdougall et al, 1999

Macdougall IC et al *J Am Soc Nephrol* 1999
Egrie & Browne *Nephrol Dial transplant* 2001

- 2002: Shire tried to launch an Epo product produced in human cells (not hamster)
  - Epoeitin delta (Dynepo)
    - **Human glycosylation pattern, less immunogenic?**
  - **Still short half-life, three doses / week**
    - **Not marketed anymore**

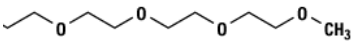
- 2007: PEGylerated Epo with much longer half-life is launched
  - PEG-epoetin beta (Mircera)
  - Total mol. weight 60 kDa
    - 30 kDa PEG conjugated to Lysine
  - **One dose / month ($T_{1/2}$ 130 h)**
  - **Patent disputes with Amgen**
    - **Not sold in the US**

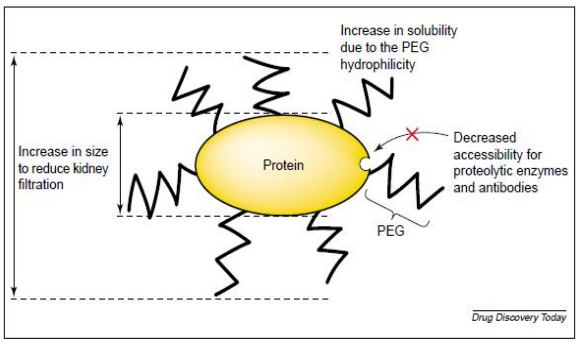
MIRCERA (Methoxy polyethylene glycol-epoetin beta)

http://www.roche.com/products/product-details.htm?type=product&id=83

PEGylation

- Covalent coupled polyethylene glycol
- Improved pharmacokinetic properties





Veronese FM 2005

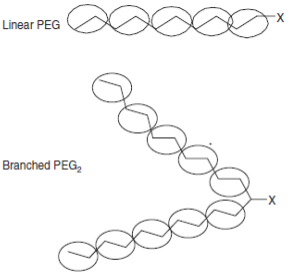


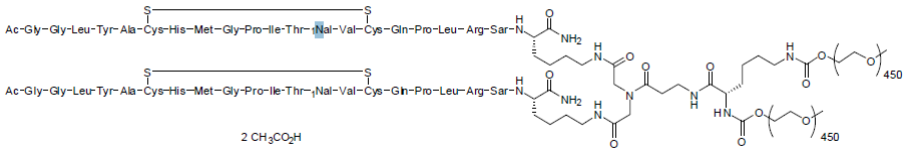
Fig. 5. Schematic representation of linear polyethylene glycol (PEG) and the branched form (PEG_2). The circles around the PEG chain represent the bound water and X represents the protein reactive groups.

Veronese FM 2008

- 2012: approval of a product that addressed both potential problems with pharmacokinetics and immunogenicity
  - Peginesatide (Omontys® Affymax)
  - Synthetic, pegylated dimeric peptide (not biotech)
    - 5 kDa peptide plus 40 kDa PEG
  - Peginesatide binds the erythropoietin receptor but does not share the Epo sequence
    - **No risk of cross-reactive immunogenicity (and PRCA)**
  - **Half-life ~50 h, 1 dose / month**

Figure 1: Structure of peginesatide acetate

Omontys peginesatide



- But in February 2013 Omontys was recalled
  - Allergic reactions (hypersensitivity), sometimes anaphylactic chock, in 0.2 % of patients

FierceBiotech
THE BIOTECH INDUSTRY'S DAILY MONITOR
NEWS TOPICS ANALYSIS FEAT

Topics:Pipeline

Omontys recall forces Affymax to ax 75% of workforce, weigh bankruptcy

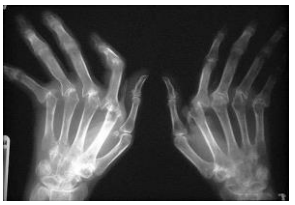
March 19, 2013 | By John Carroll

In the wake of last month's Omontys recall, Affymax ($AFFY) is jettisoning three-fourths of its staff and considering bankruptcy. Without the fuel of Omontys sales, the small drugmaker is basically running on fumes. And it has to spend money to investigate the hypersensitivity reactions--and patient deaths--that triggered the recall. So, out comes the cost-cutting ax. Report

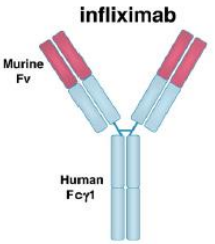
SHARE
Email
12

Example 2. TNF blockers

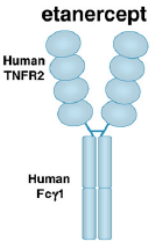
- Tumour necrosis factor (TNF) is a pro-inflammatory protein (cytokine) that signals by binding to TNF receptors
  - Involved in many inflammatory and autoimmune diseases
  - Reumatoid Arthritis (RA): chronic inflammation in joints
- Anti-TNF treatment with protein drugs since 1998
- Antibodies and antibody derived/related molecules



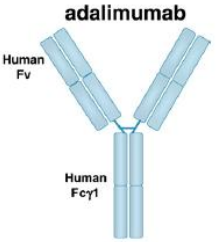
- Infliximab (Remicade, Centocor 1998)
  - Chimeric IgG1 monoclonal antibody
    - Human constant (70%) och murine variable regions (30%)
  - Produced in mouse myeloma cells (SP2/0)
  - Dosage: 3 mg/kg i.v. infusion every 6-8 week

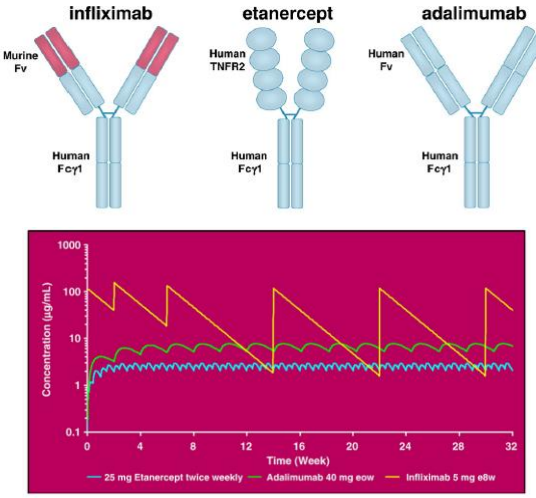


- Etanercept (Enbrel, Amgen 1998)
  - Fusion protein: extracellular parts of the TNF receptor (p75, TNFR2) and the Fc part of human IgG1.
  - Produced in CHO cells
  - Dosage: 50 mg s.c. / 1 week (or 2x /week)



- Adalimumab (Humira, Abbott 2003)
  - Fully human IgG1 mAb
    - Phage display (Cambridge Antibody Technology)
  - Produced in CHO cells
  - Dosage: 40 mg s.c. / 2 weeks

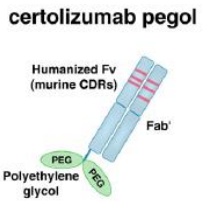




Tracey D et al. *Pharmacology & Therapeutics* 2008

36

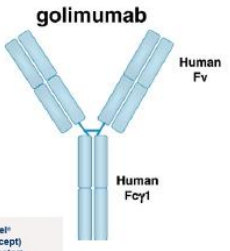
- Certolizumab pegol (Cimzia, UCB 2008)
  - Humanized antibody Fab' fragment (91 kDa), conjugated to 40kDa PEG
  - Fab' fragment is produced in *E. coli*, purified and then conjugated
  - Dosage: 400 mg s.c. / 4 weeks



http://www.cimziahcp.com/how-to-use-prefilled-syringe

- Golimumab (Simponi, Centocor 2009)
  - Fully human IgG1 mAb
    - HuMAb-Mouse (Medarex)
  - Dosage: 50 mg s.c. / 1 month

monthly Simponi™ golimumab



	SIMPONI® autoinjector + MTX¹	Humira® (adalimumab) pen¹	Cimzia® (certolizumab pegol) prefilled syringe¹	Enbrel® (etanercept) autoinjector¹
Minimum number of injections first year	12	26	28	52
Dosing schedule for adults with RA	50 mg once a month	40 mg every other week Some patients with RA not receiving MTX may benefit from increasing the frequency to 40 mg every week.	**Loading dose:** 400 mg at Weeks 0, 2, and 4 **Maintenance dose:** 200 mg every other week (400 mg every 4 weeks can be considered)	50 mg once a week

https://www.simponihcp.com/rheumatoid-arthritis/dosing

Summary: EPO and TNF examples

- Manufacturing costs
  - *E.coli* production (Certolizumab pegol)
- Immunogenicity
  - Fully human mAb (Adalimumab)
  - Allergic reactions (Peginesatide)
- Dosage and pharmacokinetics
  - Epo – normal dosage 3 injections / week
    - Hyperglycosylated: 1 dose /week; PEGylated: 1 dose /month
  - Anti-TNF
    - Golimumab: subcutaneous injection, 1 dose / month
- Intellectual property rights
  - Mircera (PEGylated Epo) infringed on Amgen patents
- Convenience
  - A subcutaneous injection that can be self-administered by the patient (e.g. on a monthly basis) using a pre-filled syringe, designed to fit a reumatic person, and that can be stored at room temperture

39

Thank you for listening

Pioneer in Rare Diseases

patrik.stromberg@sobi.com